The Journal of Nutrition
**Nutrition and Disease**

# Hypocholesterolemic Effects of Hydroxypropyl Methylcellulose Are Mediated by Altered Gene Expression in Hepatic Bile and Cholesterol Pathways of Male Hamsters[1–3]

Glenn E. Bartley,[4] Wallace Yokoyama,[4] Scott A. Young,[5] William H. K. Anderson,[5] Shao-Ching Hung,[5] David R. Albers,[5] Marsha L. Langhorst,[5] and Hyunsook Kim[4]*

[4]Processed Foods Research, Western Regional Research Center, USDA, Agricultural Research Service, Albany, CA 94710; and [5]The Dow Chemical Company, Midland, MI 48674

## Abstract

Hydroxypropyl methylcellulose (HPMC), a semisynthetic, nonfermentable soluble dietary fiber, is not absorbed by the body, but its presence in the intestinal lumen increases fecal fat, sterol, and bile acid excretions and decreases intestinal cholesterol absorption, all of which may indirectly affect hepatic lipid metabolism. We measured the expression of hepatic genes involved in cholesterol, bile acid, and fatty acid metabolism in hamsters fed diets containing 39% of energy as fat and 5% of weight as HPMC or microcrystalline cellulose (control) for 4 wk. HPMC-fed hamsters gained significantly less body weight than the control group but did not differ in food intake. They had significantly lower plasma triglyceride and total-, VLDL-, HDL-, and LDL-cholesterol concentrations and hepatic total lipid, total and free cholesterol and triglyceride concentrations than controls. Compared with controls, HPMC-fed hamsters had greater levels of mRNA for *CYP7A1* (cytochrome P450 7A1; 8-fold of control; $P < 0.05$), *CYP51* (lanosterol 14$\alpha$-demethylase; 5.3-fold of control; $P < 0.05$), and *HMG-CoAR* (3-hydroxy-3-methylglutaryl CoA reductase; 1.8-fold of control; $P < 0.05$). The plasma total cholesterol concentrations from both the control and HPMC groups were inversely correlated with expression of hepatic *CYP7A1* ($r = -0.54$; $P < 0.05$), *CYP51* ($r = -0.79$; $P < 0.005$), and *HMG-CoAR* ($r = -0.75$; $P < 0.005$) genes. This suggests that HPMC supplementation affected both cholesterol and bile acid synthesis. Our data confirm that altered hepatic expression of lipid metabolism-related genes, possibly due to modulation of fecal bile acid excretion and intestinal cholesterol absorption, contributes to the lipid-lowering effects of HPMC. J. Nutr. 140: 1255–1260, 2010.

## Introduction

Dyslipidemia is 1 of the top 5 major risk factors leading to cardiovascular disease, which is linked to ~40% of deaths in the United States (1). As an alternative to pharmacological medicine, combinations of lifestyle changes and appropriately controlled diets have recently received more attention to reduce cardiovascular disease risk. Although the hypocholesterolemic effects of natural water-soluble fibers were demonstrated >40 y ago (2), soluble dietary fiber (SDF)[6] have recently attracted renewed interest. Numerous studies have delineated the cholesterol-lowering effects of psyllium, guar gum, pectin, and $\beta$-glucan; however, the mechanisms responsible remain poorly understood. SDF increase the viscosity of small intestine contents and fecal bile acids and sterols excretion, which may in turn reduce the size of the enterohepatic pool of bile acids or possibly cause malabsorption of fats through disruption of

[1] Supported in part by a Cooperative Research and Development Agreement between The Dow Chemical Company and USDA, Agricultural Research Service, Albany, CA.

[2] Author disclosures: S. A. Young, W. H. K. Anderson, S-C. Hung, D. R. Albers, and M. L. Langhorst are employed by The Dow Chemical Company. G. E. Bartley, W. Yokoyama, and H. Kim, no conflicts of interest.

[3] Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.

* To whom correspondence should be addressed. E-mail: kimhy2005@hotmail.com.

[6] Abbreviations used: ABCG5*, ATP-binding cassette sub-family G; ACOX, acyl-CoA oxidase; CPT-1, carnitine palmitoyltransferase-1; CYP51, lanosterol 14$\alpha$-demethylase; CYP7A1, cytochrome P450 7A1; FAS, fatty acid synthase; FXR$\alpha$, farnesoid X receptor; HMG CoAR, 3-hydroxy-3-methylglutaryl CoA reductase; HNF-4$\alpha$, hepatocyte nuclear factor-4; HPMC, hydroxypropyl methylcellulose; LXR$\alpha$, liver X receptor; LDLR, LDL receptor; LRH-1, liver receptor homolog-1; MCC, microcrystalline cellulose; PGC-1, PPAR-$\gamma$ coactivator-1; SCD-1, stearoyl-CoA desaturase-1; SHP, small heterodimer partner; SDF, soluble dietary fiber; SREBP, sterol response element binding protein.


Manuscript received November 6, 2009. Initial review completed December 13, 2009. Revision accepted April 13, 2010.
First published online May 5, 2010; doi:10.3945/jn.109.118349.

intestinal micelle formation (3,4). Both of these events can result in upregulation of cholesterol synthesis. Most SDF are fermented by intestinal bacteria to produce SCFA, which can affect hepatic cholesterol synthesis (5,6).

Bile acids, synthesized from cholesterol in the liver, facilitate absorption of cholesterol, dietary lipid, and fat-soluble vitamins from the intestinal lumen. The conversion of endogenous cholesterol to bile acids and excretion of unabsorbed cholesterol and unrecycled bile acids in feces represent 2 major routes for cholesterol removal from the body. In addition to their role as a detergent to facilitate dietary fat absorption, bile acids are increasingly recognized as signaling molecules that bind to nuclear receptors such as farnesoid X receptor (FXR)$\alpha$ that regulate bile acid and cholesterol metabolism (7,8). Furthermore, altered hepatic cholesterol and oxysterol concentrations resulting from changes in bile acid synthesis can influence the function of transcription factors such as sterol response element binding protein (SREBP), whose target genes regulate the biosynthesis of cholesterol and lipids (9).

Hydroxypropyl methylcellulose (HPMC) is a well-characterized, nonfermentable, semisynthetic cellulose derivative with physiological properties of a SDF. Previous studies in animals and humans have linked increasing HPMC viscosity with hypocholesterolemic effects and increased fecal excretion of bile acids and cholesterol (10,11). These effects are similar to those of fermentable SDF such as psyllium and $\beta$-glucan. Furthermore, HPMC-supplemented diets have shown a positive effect in regulating adipocytokine production in addition to improving lipid and glucose homeostasis (12). However, the mechanism by which HPMC-mediated increased excretion of bile acids and sterols triggers the observed changes in lipid and cholesterol metabolism is not clear.

The male Syrian Golden hamster has been extensively used as an animal model to study cholesterol and bile acid metabolism, which is known to be similar to humans. In both humans and hamsters, the liver is the major site for plasma LDL-cholesterol clearance and the liver has a low rate of cholesterol synthesis (13). Less is known about the molecular events that occur concurrently with fat and fiber feeding, because the hamster genome has not been completely sequenced.

To define the mechanisms by which dietary HPMC elicits lipid (cholesterol and triglyceride)-lowering effects, we fed male Golden Syrian hamsters a high-fat diet containing either 5% HPMC or 5% microcrystalline cellulose (MCC), an inactive insoluble fiber, as a control for 4 wk and investigated hepatic gene expression profiles of key enzymes involved in cholesterol, bile acid, and fatty acid metabolism as well as nuclear receptors and transcription factors that regulate these pathways. Correlations were calculated to determine relationships between the cholesterol-lowering effects and altered hepatic gene expression for cholesterol, bile acid synthesis, and circulating adiponectin concentrations.

## Materials and Methods

*Hamsters and diets.* Male Golden Syrian hamsters (~80 g, LVG strain, Charles River) were acclimatized and given water and a 5001 rodent diet (LabDiet, PMI International; protein, 239 g/kg; fat, 50 g/kg; nonnitrogenous substances, 487 g/kg; crude fiber, 51 g/kg; ash, 70 g/kg; energy, 17 MJ/kg; and sufficient amounts of minerals and vitamins for healthy maintenance) ad libitum for 1 wk prior to the initiation of the experimental diets. Hamsters were weighed and randomized into 2 groups of 15 hamsters each and were fed high-fat diets ad libitum containing either 5% (by weight) very high viscosity HPMC (The Dow Chemical Company) or 5% MCC (Dyets) for 4 wk. MCC is an insoluble fiber that has little effect on sterol metabolism (3). Diets consisted of 18% of energy as protein, 43% as carbohydrate, and 39% as fat supplemented with 0.1% cholesterol (**Supplemental Table 1**). Body weights were recorded weekly and food intake was monitored twice per week. The study was approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA.

*Plasma and tissue collection.* Hamsters were feed deprived for 12 h and anesthetized with Isoflurane (Phoenix Pharmaceutical). Blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15% wt:v) and plasma was separated after centrifugation at 2000 $\times$ $g$ for 30 min at 4°C. Livers were collected, weighed, and immediately frozen in liquid nitrogen for analysis.

*Plasma biomarker analysis.* Cholesterol in plasma lipoproteins were determined by size-exclusion chromatography as previously described (14). Plasma triglycerides, total cholesterol, free cholesterol, and glucose were determined by enzymatic colorimetric assays using a Roche Diagnostics/Hitachi 914 Clinical Analyzer with assay kits (Roche Diagnostics and Wako Chemicals). Plasma concentrations of adiponectin (B-bridge International) and insulin (Mercodia) of feed-deprived hamsters were determined using mouse adiponectin and ultra-sensitive rat insulin immunoassay kits as previously described (12). Blood glucose concentrations in feed-deprived hamsters were measured in tail vein samples using a OneTouch Ultrameter (LifeScan).

*Hepatic lipid analysis.* Lyophilized, ground liver samples were extracted using an accelerated solvent extractor (Dionex) at 100°C, ~13.8 MPa with 75/25 hexane/2-propanol. The sample extract was analyzed on a Roche Diagnostic/Hitachi 914 clinical analyzer (Roche Diagnostics) to determine hepatic triglycerides, total cholesterol, and free cholesterol using the kits described above.

*Real-time PCR.* Total RNA from livers was extracted using TRIzol plus RNA purification kit (Invitrogen, Life Technologies) and cDNA was synthesized using GeneAmp RNA PCR kit (Applied Biosystems) per the manufacturer's protocol. Approximately 1 $\mu$L of diluted cDNA (1:10) was used in each real-time RT-PCR using SYBR Green Supermix (Bio-Rad) with an Mx3000P instrument (Stratagene). The cycle conditions were: 5 min at 95°C followed by 20–35 cycles of incubation at 94°C for 15 s, then 55–60°C for 1 min and 72°C for 30 s. The sequences of the primers used for this study are shown in **Supplemental Table 2**. The primers were validated by size and sequencing of PCR products. No accumulation of nonspecific products and primer-dimers was observed in a gel electrophoresis test of the PCR products. The results were analyzed using the software provided with the Stratagene Mx3000P QPCR system. Differences in mRNA expression were calculated after normalizing to $\beta$-*actin* expression.

*Fecal bile acids and sterol analysis.* Feces were collected for 3 consecutive days immediately prior to when the hamsters were killed and were lyophilized, milled, and stored at −20°C. Bile acids and sterols were determined by HPLC as described previously (15).

*Statistical analysis.* All data are expressed as means ± SE. Differences between control and HPMC groups were determined by 2-tailed Student's $t$ tests. When variances of each group were unequal, significance of differences was determined using the Welch's test. Pearson correlation coefficients were calculated for investigating relationships of plasma total cholesterol, plasma adiponectin concentrations, hepatic cholesterol, and triglyceride concentrations with the expression of hepatic genes (JMP 7 statistical program, SAS Institute). Significance was defined at $P < 0.05$.

## Results

*Metabolic effect of HPMC supplementation.* The final body weight and weight gain were significantly less in the HPMC

group than in the control group despite the similar food intake in both groups, resulting in a 21% lower food efficiency ratio in the HPMC group (**Table 1**). In conjunction with lower body weight, liver weight in the HPMC group was significantly lower than in the control group by 37%. The retroperitoneal adipose weight in the HPMC group tended to be ~18% lower than in the control group ($P = 0.08$). In the HPMC group, plasma total-, VLDL-, and LDL-cholesterol concentrations were 41, 70, and 60% lower, respectively, than in the control group ($P < 0.05$) (Table 1). HPMC consumption also lowered the HDL-cholesterol concentration ($P < 0.05$) and the LDL-:HDL-cholesterol ratio ($P < 0.05$). The plasma triglyceride concentration was 38% lower in HPMC group and fecal contents of bile acids and sterols were 63 and 29% greater, respectively, in the HPMC group than those in the control group (Table 1). In addition, hepatic total lipid, total cholesterol, free cholesterol, and triglyceride concentrations were 20, 73, 45, and 80% lower, respectively, in the HPMC group compared with the control group ($P < 0.05$) (Table 1).

***mRNA expression levels of hepatic genes related to bile acid and cholesterol metabolism.*** The expression of a set of hepatic genes related to bile acid metabolism was higher in the HPMC group than in the control group with the exception of *SHP-1* (small heterodimer partner), which did not differ between the groups (**Table 2**).

The mRNA levels of hepatic genes involved in cholesterol synthesis, including *SREBP-2*, *HMG-CoAR* (3-hydroxy-3-methylglutaryl CoA reductase), and *CYP51* (lanosterol 14$\alpha$-demethylase), were higher in the HPMC group compared with the control group. The expression level of *LDLR* (LDL receptor) in the HPMC group also was higher, whereas mRNA level of the sterol transporter protein, *ABCG5* (ATP-binding cassette sub-family G), in the HPMC group was lower than in the control group. However, expression levels of *LXR*$\alpha$ (liver X receptor) did not differ between the groups.

**TABLE 1** Anthropometrics and plasma lipid concentrations in male hamsters fed 5% MCC or HPMC diet for 4 wk[1]

| | 5% MCC | 5% HPMC |
|---|---|---|
| Anthropometric data | | |
| Body weight, *g* | 109.9 ± 2.0 | 101.7 ± 1.9* |
| Body weight gain, *g/4 wk* | 40.4 ± 1.9 | 32.1 ± 2.1* |
| Food intake, *g/d* | 10.6 ± 0.2 | 10.7 ± 0.3 |
| Food efficiency ratio, *g gain/g feed* | 0.35 ± 0.01 | 0.27 ± 0.02* |
| Retroperitoneal adipose tissue, *g* | 1.6 ± 0.1 | 1.3 ± 0.1 |
| Liver weight, *g* | 5.7 ± 0.2 | 3.6 ± 0.1* |
| Plasma lipids | | |
| Total cholesterol, *mmol/L* | 5.95 ± 0.21 | 3.53 ± 0.17* |
| HDL cholesterol, *mmol/L* | 2.80 ± 0.09 | 2.37 ± 0.09* |
| VLDL cholesterol, *mmol/L* | 1.08 ± 0.09 | 0.33 ± 0.05* |
| LDL cholesterol, *mmol/L* | 1.88 ± 0.15 | 0.83 ± 0.07* |
| LDL:HDL cholesterol ratio | 0.75 ± 0.06 | 0.35 ± 0.03 * |
| Triglyceride, *mmol/L* | 2.11 ± 0.33 | 1.31 ± 0.14* |
| Fecal bile acids and sterols, *μmol/g* | | |
| Fecal bile acids | 1.8 ± 0.2 | 5.0 ± 0.7* |
| Fecal sterols | 10.9 ± 0.6 | 15.3 ± 1.0* |
| Hepatic lipids | | |
| Percent total lipid, *g/100 g* | 21.4 ± 0.6 | 17.2 ± 1.0* |
| Total cholesterol, *μmol/g* | 89.6 ± 4.0 | 24.3 ± 2.1* |
| Free cholesterol, *μmol/g* | 31.9 ± 2.0 | 17.4 ± 1.2* |
| Triglyceride, *μmol/g* | 69.6 ± 2.0 | 13.3 ± 2.1* |

[1] Values are means ± SE, $n = 15$. *Different from MCC, $P < 0.05$.

**TABLE 2** Plasma adiponectin concentration and hepatic mRNA expression of genes related to homeostasis of bile acids, cholesterol, and fatty acid metabolism in male hamsters fed 5% MCC or HPMC diet for 4 wk[1]

| | 5% MCC | 5% HPMC |
|---|---|---|
| Plasma adiponectin, *mg/L* | 22.3 ± 1.72 | 30.9 ± 1.60 |
| Relative gene expression | | |
| Bile acid synthesis-related genes | | |
| *FXR α* | 1.07 ± 0.16 | 2.45 ± 0.27* |
| *SHP-1* | 1.14 ± 0.25 | 1.42 ± 0.30 |
| *LRH-1* | 1.04 ± 0.14 | 1.63 ± 0.13* |
| *HNF-4α* | 1.03 ± 0.09 | 1.80 ± 0.29* |
| *PGC-1α* | 1.02 ± 0.06 | 1.61 ± 0.23* |
| *CYP7A1* | 1.07 ± 0.18 | 8.34 ± 1.82* |
| *ABCB11* | 1.06 ± 0.14 | 2.50 ± 0.31* |
| Cholesterol synthesis-related genes | | |
| *LXR α* | 1.03 ± 0.11 | 1.38 ± 0.14 |
| *SREBP-2* | 1.04 ± 0.10 | 2.71 ± 0.64* |
| *HMG-CoAR* | 1.04 ± 0.12 | 1.84 ± 0.21* |
| *CYP51* | 1.05 ± 0.11 | 5.54 ± 0.99* |
| *LDLR* | 1.02 ± 0.09 | 2.31 ± 0.28* |
| *ABCG5* | 1.03 ± 0.10 | 0.39 ± 0.05* |
| Fatty acid metabolism-related genes | | |
| *PPAR α* | 1.03 ± 0.10 | 1.77 ± 0.29* |
| *ACOX* | 1.06 ± 0.15 | 2.84 ± 0.41* |
| *CPT-1* | 1.09 ± 0.20 | 2.14 ± 0.28* |
| *SREBP-1c* | 1.05 ± 0.11 | 0.59 ± 0.07* |
| *SCD-1* | 1.08 ± 0.07 | 0.30 ± 0.09* |
| *FAS* | 1.04 ± 0.10 | 0.65 ± 0.12* |

[1] Values are means ± SE, $n = 8$–10. *Different from MCC, $P < 0.05$. Each mRNA was normalized to β-actin and is expressed as a relative level.

***mRNA expression levels of hepatic genes related to lipid metabolism.*** Expression levels of *PPAR*$\alpha$, a transcription factor regulating fatty acid β-oxidation and its target genes, *ACOX* (acyl-CoA oxidase) and *CPT-1* (carnitine palmitoyl-transferase-1), encoding genes for rate-limiting enzymes for mitochondrial and peroxisomal fatty acid oxidation, were greater in the HPMC group than in the control group. In contrast, a transcription factor for fatty acid synthesis, *SREBP-1c*, and genes encoding for key enzymes for fatty acid synthesis, *SCD-1* (stearoyl-CoA desaturase-1) and *FAS* (fatty acid synthase), were lower in the HPMC group compared with the control group (Table 2).

***Plasma adiponectin, insulin, and glucose concentrations.*** The plasma adiponectin concentration was 31% greater in the HPMC group than in the control group ($P < 0.05$; Table 2). However, plasma insulin concentrations measured following feed deprivation did not differ between the control (13.8 ± 5.2 pmol/L) and HPMC (17.2 ± 5.2 pmol/L) groups. Similarly, glucose concentrations did not differ between the control (5.72 ± 0.29 mmol/L) and HPMC (5.83 ± 0.28 mmol/L) groups.

***Correlation between plasma lipid and adiponectin concentrations and hepatic gene expression.*** Correlations of plasma cholesterol concentrations, hepatic triglycerides, and hepatic gene expression levels were examined to understand the relationships between molecular processes and circulating lipid concentrations (**Table 3**). Total-, VLDL-, LDL-, and HDL-cholesterol were negatively correlated with hepatic expression of

**TABLE 3** Correlations between plasma cholesterol concentrations and expression of hepatic genes in male hamsters fed 5% MCC and HPMC diets for 4 wk[1]

| Genes | Total cholesterol | VLDL cholesterol | LDL cholesterol | HDL cholesterol |
|---|---|---|---|---|
| | | | *r* | |
| *CYP7A1* | −0.54* | −0.52* | −0.36 | −0.64** |
| *CYP51* | −0.79*** | −0.73*** | −0.70*** | −0.64*** |
| *HMG-CoAR* | −0.75*** | −0.68*** | −0.61*** | −0.71*** |
| *ABCG5* | 0.77*** | 0.60* | 0.75*** | 0.62* |
| *ABCB11* | −0.71*** | −0.60* | −0.61* | −0.66** |
| *LDLR* | −0.76*** | −0.64** | −0.67** | −0.70*** |

[1] Values are Pearson correlation, $n = 16$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.

*CYP7A1* (cytochrome P450 7A1), *CYP51*, *HMG-CoAR*, *ABCB11*, and *LDLR* genes. There were significant positive correlations between plasma cholesterol concentrations (total-, VLDL-, LDL-, and HDL-cholesterol) and mRNA levels of the cholesterol transporter, *ABCG5*. Moreover, the mRNA levels of *SREBP-2* were positively correlated with *LDLR* ($r = 0.75$; $P < 0.01$), *CYP51* ($r = 0.68$; $P < 0.01$), and *HMG-CoAR* ($r = 0.83$; $P < 0.01$) mRNA levels. Positive correlations between hepatic triglyceride concentrations and mRNA levels of *SREBP-1* ($r = 0.71$; $P < 0.01$) and *SCD-1* ($r = 0.85$; $P < 0.01$) were observed. Furthermore, there were negative correlations between hepatic triglyceride concentrations and mRNA levels of *PPARα* ($r = -0.54$; $P < 0.05$) and *ACOX* ($r = -0.74$; $P < 0.01$) mRNA levels. In addition, plasma adiponectin concentrations were negatively correlated to hepatic triglyceride concentrations ($r = -0.66$; $P < 0.01$).

## Discussion

HPMC and other SDF have a positive impact on dyslipidemia in both humans and animal models. No single mechanism linking these macro-observations with control of the body's biochemistry has been identified. In this hamster study, we correlate the upregulation of hepatic pathways for bile acid and cholesterol synthesis and downregulation of triglyceride synthesis with the physiological consequences of HPMC dietary supplementation.

Hepatic gene expression data suggest that HPMC's cholesterol-lowering effect is a consequence of increased loss of bile acids to the feces. Therefore, hepatic cholesterol is directed into bile acid synthesis to compensate for the decreased bile acid concentrations in the enterohepatic circulation, as suggested by the increase in the expression of *CYP7A1* mRNA (**Fig. 1**). This is accompanied by an upregulation of hepatic cholesterol synthesis as implied by increases in expression of *CYP51* and *HMG-CoAR* mRNA. These data are supported by significant negative correlations between plasma cholesterol concentrations and the relative expression of *CYP7A1*, *CYP51*, and *HMG-CoAR* mRNA levels. Changes in mRNA expression levels are not always a measure of pathway flux; however, previous studies have shown a linear relationship between enzyme activity and gene expression for many of these enzymes. For example, dietary supplementation with psyllium or cholestyramine upregulates both *CYP7A1* mRNA and CYP7A1 enzyme activity (3,16).

We also measured similar changes to critical regulatory genes. Upregulation of *SREBP-2* and its downstream target genes such as *LDLR* and *CYP51* and *HMG-CoAR* (17) showed a strong positive correlation. A strong negative correlation between the expression of *LDLR* and plasma cholesterol fits with these observations. We hypothesize that bile acid synthesis demand for cholesterol upregulates not only de novo cholesterol synthesis through *CYP51* and *HMG-CoAR* expression but also optimizes cholesterol transport by increasing *LDLR* gene expression and the import of exogenous cholesterol. Additionally, downregulation of *ABCG5*, which would limit cholesterol efflux to the bile duct, was observed. *ABCG5* is a target gene of LXRα, which regulates cholesterol metabolism and transport upon activation by cholesterol derivatives such as (24S), 25-epoxycholesterol, (24S)- and (22R)-hydroxycholesterol (18). Even though no significant change in the expression of *LXRα* was observed under these conditions in hamsters, less activated LXRα due to the low hepatic cholesterol concentrations in HPMC group may lead to downregulation of *ABCG5*. Consistent with the lower *ABCG5* expression, another target gene of LXRα, *SREBP-1c*, was also downregulated by HPMC.

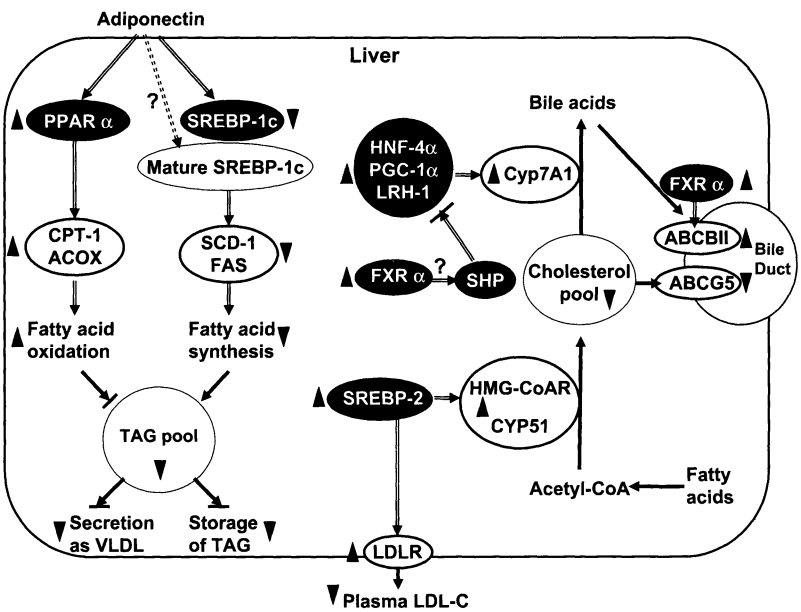


**FIGURE 1** Summary of the proposed mechanism responsible for lipid-lowering effects in hamsters fed a high-fat diet containing HPMC. Replacement of lost bile acids from their precursor cholesterol is seen in the upregulation (▲) or downregulation (▼) of a number of genes for transcription factors (●), and enzymes or proteins (○) in the HPMC group relative to the control group. These genes are critical to the control of the biosynthetic pathways and in the transport of bile acids and cholesterol across hepatocyte membranes. Increased plasma adiponectin concentration may modulate expression of genes related to fatty acid synthesis and oxidation and reduce plasma and hepatic triglyceride concentration.

As the rate-limiting step in the conversion of cholesterol to bile acids, *CYP7A1* plays an important role in the cholesterol-lowering effect of HPMC; however, several nuclear receptors can be involved in its regulation, including LXR$\alpha$, FXR$\alpha$, SHP-1, liver receptor homolog-1 (LRH-1), and hepatocyte nuclear factor-4 (HNF4$\alpha$) (7). The present results indicate that the upregulation of *CYP7A1* by HPMC is not associated with the LXR$\alpha$-mediated pathway, because decreased mRNA expression would be expected for LXR$\alpha$ target genes. FXR$\alpha$ regulates bile acid synthesis and enterohepatic circulation of bile acids upon activation by bile acids. Activated FXR$\alpha$ induces *ABCB11*, which codes for BSEP and *SHP-1*, whose product inhibits *CYP7A1* transcription (19). However, the 2 FXR$\alpha$ target genes, *SHP-1* and *ABCB11*, had inconsistent mRNA expression in hamster liver. The increased mRNA expression of *ABCB11* by HPMC reflects the activation of FXR$\alpha$ and would cause increased efflux of bile acids; however, no significant changes were observed for *SHP-1* expression levels. This might be explained by the rapid and transient induction of *SHP-1*, so increased expression levels of *SHP-1* may have been missed because of the time of liver specimen sampling (7). Therefore, upregulation of *CYP7A1* in hamster liver may not be associated with the *FXR/SHP* regulation pathway. Alternatively, FXR$\alpha$-independent pathways for the regulation of *CYP7A1* involving HNF-4$\alpha$ have been reported (20). *HNF-4$\alpha$* and *LRH-1* gene products bind to the *CYP7A1* promoter and HNF-4$\alpha$ with PPAR-$\gamma$ coactivator-1 (PGC-1$\alpha$) increase basal activation of *CYP7A1* and increase bile acid production (21,22). Increased expression of *HNF-4$\alpha$*, *LRH-1*, and *PGC-1$\alpha$* was significantly enhanced with HPMC supplementation, indicating that the bile acid biosynthesis may be regulated by HNF-4$\alpha$. In addition to the hepatic nuclear receptors examined in this study, intestinal *FGF-15* (*FGF-19* in mice) has been reported as an FXR target gene (23). Further studies regarding the physiological relationship of these factors or nuclear receptors are needed to bring more insights for the understanding of enterohepatic regulation of bile acid synthesis.

Adiponectin is an adipocyte-secreted protein that has been shown to increase fatty acid oxidation (24). We confirm here an earlier observation from our laboratories that HPMC supplementation of a high-fat diet in hamsters leads to an increase in plasma adiponectin concentrations that are correlated with reductions in plasma cholesterol ($r = -0.41$; $P = 0.02$) and triglyceride concentrations ($r = -0.26$; $P = 0.16$) (12). An association of plasma adiponectin concentrations with increased expression of *SREBP-1c* and decreased expression of *PPAR$\alpha$* and their downstream genes has been reported, linking regulation of the hepatic triglyceride pool with plasma adiponectin concentrations (25). We have confirmed and extended those observations by showing upregulation of *PPAR$\alpha$*, *CPT-1*, and *ACOX* and downregulation of *SREBP-1c*, *SCD-1*, and *FAS*. Furthermore, significant correlations between hepatic triglyceride, plasma adiponectin concentrations, and *SREBP-1c*, *SCD-1*, *PPAR$\alpha$*, and *ACOX* expression levels were observed.

In summary, we have demonstrated that the improvements in plasma lipid profiles in hamsters fed a high-fat diet supplemented with the SDF, HPMC, are linked to the regulation of bile acid, cholesterol, and triglyceride metabolism in the liver through increases in fecal bile acid excretion. Cholesterol and bile acid transport in and out of hepatocytes is also modified. Plasma and hepatic triglyceride concentrations were reduced by modulating expression of genes related to fatty acid synthesis and oxidation, which may be mediated by increased circulating adiponectin concentrations. This study further supports the potential dietary use of HPMC for the prevention or management of dyslipidemia-related diseases such as cardiovascular disease, metabolic syndrome, and possibly obesity.


### Acknowledgments

We thank Drs. Manabu T. Nakamura (University of Illinois at Urbana-Champaign) and Paul Davis (University of California, Davis) for manuscript editing, Maggie Chiu for blood and tissue preparation, and Wanless Hatcher and Ezekial Martinez for their diligent attention to animal care. G.B., W.Y., S.C.H., D.R.A., M.L.L., and H.K. conducted research; W.Y., S.A.Y., W.H.K.A., and H.K. designed research; H.K., W.Y., S.A.Y., W.H.K.A., and S. H., wrote the paper; G.B., W.Y., S.H., D.R.A., M.L.L., and H. K. analyzed data; and H.K., W.Y., S.A.Y., and W.H.K.A. had primary responsibility for final content. All authors read and approved the final manuscript.


## Literature Cited


1. Albert MA. Inflammatory biomarkers, race/ethnicity and cardiovascular disease. Nutr Rev. 2007;65:S234–8.
2. Ershoff BH, Wells AF. Effects of gum guar, locust bean gum and carrageenan on liver cholesterol of cholesterol fed rats. Proc Soc Exp Biol Med. 1962;110:580–2.
3. Horton JD, Cuthbert JA, Spady DK. Regulation of hepatic 7 alpha-hydroxylase expression by dietary psyllium in the hamster. J Clin Invest. 1994;93:2084–92.
4. Moriceau S, Besson C, Levrat MA, Moundras C, Rémésy C, Morand C, Demigné C. Cholesterol-lowering effects of guar gum: changes in bile acid pools and intestinal reabsorption. Lipids. 2000;35:437–44.
5. Matheson HB, Story JA. Dietary psyllium hydrocolloid and pectin increase bile acid pool size and change bile acid composition in rats. J Nutr. 1994;124:1161–5.
6. Gallaher DD, Hassel CA, Lee KJ, Gallaher CM. Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect in hamsters. J Nutr. 1993;123:244–52.
7. Chiang JY. Bile acids: regulation of synthesis. J Lipid Res. 2009;50: 1955–66.
8. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev. 2009;89:147–91.
9. Radhakrishnan A, Ikeda Y, Kwon HJ, Brown MS, Goldstein JL. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig. Proc Natl Acad Sci USA. 2007;104:6511–8.
10. Maki KC, Davidson MH, Torri S, Ingram KA, O'Mullane J, Daggy BP, Albrecht HH. High-molecular-weight hydroxypropylmethylcellulose taken with or between meals is hypocholesterolemic in adult men. J Nutr. 2000;130:1705–10.
11. Maki KC, Carson ML, Kerr Anderson WH, Geohas J, Reeves MS, Farmer MV, Turowski M, Miller M, Kaden VN, et al. Lipid-altering effects of different formulations of hydroxypropylmethylcellulose. J Clin Lipidol. 2009;3:159–66.
12. Hung SC, Bartley G, Young SA, Albers DR, Dielman DR, Anderson WHK, Yokoyama W. Dietary fiber improves lipid homeostasis and modulates adipocytokines in hamsters. J Diabetes. 2009;1:194–206.
13. Turley SD, Spady DK, Dietschy JM. Role of liver in the synthesis of cholesterol and the clearance of low density lipoproteins in the cynomolgus monkey. J Lipid Res. 1995;36:67–79.
14. German JB, Xu R, Walzem R, Kinsella JE, Knuckles B, Nakamura M, Yokoyama WH. Effect of dietary fats and barley fiber on total cholesterol and lipoprotein cholesterol distribution in plasma of hamsters. Nutr Res. 1996;16:1239–49.
15. Hong YJ, Turowski M, Lin JT, Yokoyama WH. Simultaneous characterization of bile acid, sterols, and determination of acylglycerides in feces from soluble cellulose-fed hamsters using HPLC with evaporative light-scattering detection and APCI–MS. J Agric Food Chem. 2007;55:9750–7.

16. Suckling KE, Benson GM, Bond B, Gee A, Glen A, Haynes C, Jackson B. Cholesterol lowering and bile acid excretion in the hamster with cholestyramine treatment. Atherosclerosis. 1991;89:183–90.
17. Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, Goldstein JL. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc Natl Acad Sci USA. 2003;100:12027–32.
18. Yu L, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, Hobbs HH. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J Clin Invest. 2002;110:671–80.
19. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. J Biol Chem. 2001;276:28857–65.
20. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol Cell. 2000;6:507–15.
21. Abrahamsson A, Gustafsson U, Ellis E, Nilsson LM, Sahlin S, Björkhem I, Einarsson C. Feedback regulation of bile acid synthesis in human liver: importance of HNF-4 for regulation of CYP7A1. Biochem Biophys Res Commun. 2005;330:395–9.
22. Chiang JYL. Bile acid regulation of hepatic physiology: III. Bile acids and nuclear receptors. Am J Physiol Gastrointest Liver Physiol. 2003;284:G349–56.
23. Holt JA, Luo G, Billin AN, Bisi J, McNeill Y, Kozarsky KF, Donahee M, Wang DY, Mansfield TA, et al. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. Genes Dev. 2003;17:1581.
24. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med. 2002;8:1288–95.
25. Zhu M, Miura J, Lu LX, Bernier M, DeCabo R, Lane MA, Roth GS, Ingram DK. Circulating adiponectin levels increase in rats on caloric restriction: the potential for insulin sensitization. Exp Gerontol. 2004;39:1049–59.